E.Z.N.A.® Blood DNA Mini Kit

D3392-00	5 preps
D3392-01	50 preps
D3392-02	200 preps

May 2013

E.Z.N.A.® Blood DNA Mini Kit

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Manual Revision: May 2013

OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction and Overview

The E.Z.N.A.® family of products is an innovative system that radically simplifies the extraction and purification of nucleic acids from a variety of sources. The key to this system is the new HiBind® matrix that specifically, but reversibly, binds DNA or RNA under optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or a low salt buffer.

The E.Z.N.A.® Blood DNA Mini Kit provides an easy and rapid method for the isolation of genomic DNA for consistent PCR and Southern analysis. Up to 250 µL of fresh, frozen, or anticoagulated whole blood can be readily processed at one time. The E.Z.N.A.® Blood DNA Mini Kit can also be used for the preparation of genomic DNA from buffy coat, serum, plasma, saliva, buccal swabs, and other body fluids. The E.Z.N.A.® Blood DNA Kit allows for single or multiple simultaneous processing of multiple samples. There is no need for phenol/chloroform extractions, and time-consuming steps are eliminated (e.g. precipitation using isopropanol or ethanol). Purified DNA obtained with the E.Z.N.A.® Blood DNA Kit is ready for applications such as PCR, restriction digestion, and Southern blotting.

Benefits of the E.Z.N.A.® Blood DNA Mini Kit

- Optimized buffers that guarantee pure DNA
- No organic extractions
- Purified DNA can be directly used in most downstream applications

Binding Capacity

Each HiBind® DNA Mini Column can bind approximately 100 µg DNA. Using greater than 250 µL whole blood or buffy coat is not recommended.

New in this Edition:

- HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.
- Equilibration Buffer (used in the Troubleshooting section) is no longer included with this kit.
- Equilibration Buffer can be replaced with 3M NaOH provided by the user.
- OB Protease is now supplied in a liquid form eliminating the resuspension step to prior to use.
- OB Protease Solution can also be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.

Illustrated Protocol

Kit Contents

Product	D3392-00	D3392-01	D3392-02
Purifications	5 preps	50 preps	200 preps
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tube	15	150	600
BL Buffer	5 mL	20 mL	60 mL
HBC Buffer	4 mL	25 mL	80 mL
DNA Wash Buffer	1.5 mL	15 mL	3 x 25 mL
Elution Buffer	5 mL	40 mL	160 mL
OB Protease Solution	150 µL	1.5 mL	6 mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® Blood DNA Mini Kit components are guaranteed for 12 months from the date of purchase when stored as follows. OB Protease Solution can be stored at room temperature for 12 months. For long-term storage (>12 months), store OB Protease Solution at 2-8°C. All other components should be stored at room temperature. During shipment or storage under cool ambient conditions, a precipitate may form in the BL Buffer. If a precipitate is present, heat the bottle at 37°C to dissolve.

Preparing Reagents

1. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D3392-00	6 mL
D3392-01	60 mL
D3392-02	100 mL per bottle

2. Dilute HBC Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D3392-00	1.6 mL
D3392-01	10 mL
D3392-02	32 mL

E.Z.N.A.® Blood DNA Mini Kit Protocol - Blood and Body Fluids

The procedure below has been optimized for the use with fresh or frozen blood samples up to 250 µL in volume. Anti-coagulated blood, saliva, serum, buffy coat, or other body fluids can also be used. In addition, ≤ 10^7 of leukocytes or cultured cells may be used with this procedure. For DNA extraction from tissue and mouse tail we suggest that you use the E.Z.N.A.® Tissue DNA Kit (Product No. D3396). To isolate viral RNA from serum or other non-cellular body fluids the E.Z.N.A.® Viral RNA Kit (Product No. R6874) is recommended.

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 2 mL microcentrifuge tubes
- Water bath, incubator, or heat block capable of 65°C
- 100% Ethanol
- Isopropanol
- Optional: 10mM Tris-HCl or PBS
- Optional: RNase stock solution (when RNA-free genomic DNA is required)

Before Starting:

- Prepare DNA Wash Buffer and HBC Buffer according to the directions in the "Preparing Reagents" section on Page 5.
- Heat the Elution Buffer to 65°C.

1. Transfer the sample into a sterile microcentrifuge tube and bring the volume up to 250 µL with 10mM Tris-HCl, PBS, or Elution Buffer (provided).

2. Add 25 µL OB Protease Solution and 250 µL BL Buffer. Vortex at maximum speed for 15 seconds.

   Optional: If RNA-free genomic DNA is required, add 5 µL RNase A (50 mg/mL).

3. Incubate at 65°C for 10 minutes. Vortex briefly once during incubation.

4. Add 260 µL 100% ethanol. Vortex at maximum speed for 20 seconds.

5. Centrifuge briefly to collect any drops from the inside of the lid.

6. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

7. Transfer the entire sample to the column.

8. Centrifuge at ≥10,000 x *g* for 1 minute.

9. Discard the filtrate and the Collection Tube.

10. Insert the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

11. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 5 for instructions.

12. Centrifuge at ≥10,000 x *g* for 1 minute.

13. Discard the filtrate and reuse Collection Tube.

14. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol according to the instructions in the "Preparing Reagents" section on Page 5.

15. Centrifuge at 10,000 x *g* for 1 minute.

16. Discard the filtrate and reuse the Collection Tube.

17. Repeat Steps 14-16 for a second DNA Wash Buffer wash step.

18. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed (≥10,000 x *g*) to dry the column matrix.

    Note: It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

19. Transfer the HiBind® DNA Mini Column into a nuclease-free 2 mL microcentrifuge tube.

20. Add 100-200 µL Elution Buffer heated to 65°C.

21. Let sit at room temperature for 5 minutes.

    Note: Incubating the HiBind® DNA Mini Column at 65°C rather than room temperature will give a modest increase in DNA yield per elution.

22. Centrifuge at ≥13,000 x *g* for 1 minute.

23. Repeat Steps 20-22 for a second elution step.

    Note: Any combination of the following steps can be used to help increase DNA yield.

    - After adding the Elution Buffer, incubate the column for 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Elution Buffer (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

24. Store DNA at -20°C.

E.Z.N.A.® Blood DNA Mini Kit Protocol - Buccal Swabs

This protocol requires an increased volume of BL Buffer. Fewer preparations can be performed. Additional BL Buffer can be purchased separately.

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 2 mL microcentrifuge tubes
- Water bath, incubator, or heat block capable of 65°C
- 100% Ethanol
- Isopropanol
- PBS
- Optional: RNase stock solution (when RNA-free genomic DNA is required)

Before Starting:

- Prepare DNA Wash Buffer and HBC Buffer according to the directions in the "Preparing Reagents" section on Page 5.
- Heat the Elution Buffer to 65°C.

1. Place the Buccal Swab in a 2 mL microcentrifuge tube.

2. Add 500 µL PBS.

   Optional: If RNA-free genomic DNA is required, add 5 µL RNase A (50 mg/mL).

3. Add 25 µL OB Protease Solution and 500 µL BL Buffer. Vortex at maximum speed for 30 seconds.

4. Incubate at 65°C for 10 minutes.

5. Discard the buccal swab.

6. Add 500 µL 100% ethanol. Vortex at maximum speed for 20 seconds.

7. Centrifuge briefly to collect any drops from the inside of the lid.

8. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

9. Transfer 750 µL sample to the column.

10. Centrifuge at 10,000 x *g* for 1 minute.

11. Discard the filtrate and reuse the Collection Tube.

12. Repeat Steps 9-11 until all the sample has been transferred to the column.

13. Place the HiBind DNA Mini Column into a new 2 mL Collection Tube.

14. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 5 for instructions.

15. Centrifuge at ≥10,000 x *g* for 1 minute.

16. Discard the filtrate and reuse Collection Tube.

17. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol according to the instructions in the "Preparing Reagents" section on Page 5.

18. Centrifuge at 10,000 x *g* for 1 minute.

19. Discard the filtrate and reuse the Collection Tube.

20. Repeat Steps 17-19 for a second DNA Wash Buffer wash step.

21. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed (≥10,000 x *g*) to dry the column matrix.

    Note: It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

22. Transfer the HiBind® DNA Mini Column into a nuclease-free 2 mL microcentrifuge tube.

23. Add 100-200 µL Elution Buffer heated to 65°C.

24. Let sit at room temperature for 5 minutes.

    Note: Incubating the HiBind® DNA Mini Column at 65°C rather than room temperature will give a modest increase in DNA yield per elution.

25. Centrifuge at ≥13,000 x *g* for 1 minute.

26. Repeat Steps 23-25 for a second elution step.

    Note: Any combination of the following steps can be used to help increase DNA yield.

    - After adding the Elution Buffer, incubate the column for 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Elution Buffer (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

27. Store DNA at -20°C.

E.Z.N.A.® Blood DNA Mini Kit Protocol - Dried Blood

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 2 mL microcentrifuge tubes
- Water bath, incubator, or heat block capable of 65°C
- 100% Ethanol
- Isopropanol
- PBS
- Optional: RNase stock solution (when RNA-free genomic DNA is required)

Before Starting:

- Prepare DNA Wash Buffer and HBC Buffer according to the directions in the "Preparing Reagents" section on Page 5.
- Heat the Elution Buffer to 65°C.

1. Cut or punch-out the blood spot from the filter paper (up to 200 µL blood can be used per spot). Tear or cut the filter paper into small pieces and place them into a 2 mL nuclease-free microcentrifuge tube.

2. Add 250 µL PBS.

3. Incubate at 65°C for 1 hour. Vortex briefly every 20 minutes.

4. Add 25 µL OB Protease Solution. Vortex at maximum speed for 15 seconds.

5. Incubate at 65°C for 30 minutes. Vortex briefly several times during incubation.

6. Centrifuge at ≥13,000 x *g* for 5 minutes.

7. Transfer the supernatant to a nuclease-free 2 mL microcentrifuge tube.

8. Add 1 volume BL Buffer and 1 volume 100% ethanol. Vortex to mix thoroughly.

9. Centrifuge briefly to collect any drops from the inside of the lid.

10. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

11. Transfer the entire sample to the column.

12. Centrifuge at ≥10,000 x *g* for 1 minute.

13. Discard the filtrate and the Collection Tube.

14. Insert the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

15. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 5 for instructions.

16. Centrifuge at ≥10,000 x *g* for 1 minute.

17. Discard the filtrate and reuse Collection Tube.

18. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol according to the instructions in the "Preparing Reagents" section on Page 5.

19. Centrifuge at 10,000 x *g* for 1 minute.

20. Discard the filtrate and reuse the Collection Tube.

21. Repeat Steps 18-20 for a second DNA Wash Buffer wash step.

22. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed (≥10,000 x *g*) to dry the column matrix.

    Note: It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

23. Transfer the HiBind® DNA Mini Column into a nuclease-free 2 mL microcentrifuge tube.

24. Add 100-200 µL Elution Buffer heated to 65°C.

25. Let sit at room temperature for 5 minutes.

    Note: Incubating the HiBind® DNA Mini Column at 65°C rather than room temperature will give a modest increase in DNA yield per elution.

26. Centrifuge at ≥13,000 x *g* for 1 minute.

27. Repeat Steps 24-26 for a second elution step.

    Note: Any combination of the following steps can be used to help increase DNA yield.

    - After adding the Elution Buffer, incubate the column for 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Elution Buffer (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

28. Store DNA at -20°C.

    Note: Blood spots from finger pricks usually contain no more than 50 µL blood, and yield approximately 500 ng to 1 µg DNA. This is usually sufficient for PCR analysis. To obtain higher DNA concentrations, elute with 50 µL preheated elution buffer (volumes lower than 50 µL greatly reduce yields). Alternatively, the first eluate can be used to perform a second elution.

E.Z.N.A.® Blood DNA Mini Kit Protocol - Buffy Coat

The buffy coat fraction of whole blood is enriched with leukocytes and usually gives at least 5-fold more DNA than the same volume of blood. To prepare the buffy coat from fresh whole blood, simply centrifuge the sample at 3,000-4,000 x *g* for 10 minutes at room temperature. Three layers should form: a plasma upper layer, a buffy coat middle layer, and an erythrocyte bottom layer. Carefully aspirate the plasma, making sure not to disturb the layer of concentrated leukocytes. The buffy coat can be drawn off with a pipette and used directly in the E.Z.N.A.® Blood DNA Mini Kit or frozen at -70°C.

This protocol requires an increased volume of BL Buffer. Fewer preparations can be performed. Additional BL Buffer can be purchased separately.

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 2 mL microcentrifuge tubes
- Water bath, incubator, or heat block capable of 65°C
- 100% Ethanol
- Isopropanol
- Optional: 10mM Tris-HCl or PBS
- Optional: RNase stock solution (when RNA-free genomic DNA is required)

Before Starting:

- Prepare DNA Wash Buffer and HBC Buffer according to the directions in the "Preparing Reagents" section on Page 5.
- Heat the Elution Buffer to 65°C.

1. Transfer the sample into a sterile microcentrifuge tube and bring the volume up to 500 µL with 10mM Tris-HCl, PBS, or Elution Buffer (provided).

2. Add 25 µL OB Protease Solution and 500 µL BL Buffer. Vortex at maximum speed for 15 seconds.

   Optional: If RNA-free genomic DNA is required, add 2 µL RNase A (50 mg/mL).

3. Incubate at 65°C for 10 minutes. Vortex briefly once during incubation.

4. Add 500 µL 100% ethanol. Vortex at maximum speed for 20 seconds.

5. Centrifuge briefly to collect any drops from the inside of the lid.

6. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

7. Transfer the 750 µL sample to the column.

8. Centrifuge at ≥10,000 x *g* for 1 minute.

9. Discard the filtrate and the Collection Tube.

10. Repeat Steps 7-9 until all the sample has been transferred to the column.

11. Insert the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

12. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 5 for instructions.

13. Centrifuge at ≥10,000 x *g* for 1 minute.

14. Discard the filtrate and reuse Collection Tube.

15. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol according to the instructions in the "Preparing Reagents" section on Page 5.

16. Centrifuge at 10,000 x *g* for 1 minute.

17. Discard the filtrate and reuse the Collection Tube.

18. Repeat Steps 15-17 for a second DNA Wash Buffer wash step.

19. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed (≥10,000 x *g*) to dry the column matrix.

    Note: It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

20. Transfer the HiBind® DNA Mini Column into a nuclease-free 2 mL microcentrifuge tube.

21. Add 100-200 µL Elution Buffer heated to 65°C.

22. Let sit at room temperature for 5 minutes.

    Note: Incubating the HiBind® DNA Mini Column at 65°C rather than room temperature will give a modest increase in DNA yield per elution.

23. Centrifuge at ≥13,000 x *g* for 1 minute.

24. Repeat Steps 21-23 for a second elution step.

    Note: Any combination of the following steps can be used to help increase DNA yield.

    - After adding the Elution Buffer, incubate the column for 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Elution Buffer (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

25. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Clogged Column	Incomplete Lysis	Extend incubation time with BL Buffer and OB Protease Solution.
	Too much Sample	Divide sample into multiple tubes and adjust the volume to 250 µL with BL Buffer.
	Sample is Viscous	Divide sample into multiple tubes and adjust the volume to 250 µL with BL Buffer.
Problem	**Cause**	**Solution**
Low DNA Yield	Poor Elution	Repeat elution with increased elution volume. Incubate columns at 65°C for 5 minutes with Elution Buffer.
	Improper Washing	DNA Wash Buffer must be diluted with 100% ethanol before use. If refrigerated, DNA Wash Buffer must be brought to room temperature.
		HBC Buffer must be diluted with isopropanol before use. If refrigerated, HBC Buffer must be brought to room temperature.
	Sample has low DNA Content	Increase starting material and volume of all reagents (OB Protease, BL Buffer, ethanol) proportionally. Load aliquots of lysate through column successively.
	Prime Columns	Add 100 µL 3M NaOH to the column prior to loading the sample. Let sit for 4 minutes. Centrifuge at 10,000 x *g* for 60 seconds. Add 100 µL water to the column and centrifuge at 10,000 x *g* for 60 seconds. Discard the filtrate.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
2 mL DNase/RNase-free Microcentrifuge Tubes, 1000/pk, 10 pk/cs	SSI-1310-00
BL Buffer, 100 mL	PD062
DNA Wash Buffer, 100 mL	PS010
Elution Buffer, 100 mL	PDR048
RNase A, 100 mg/mL, 2.5 mL	RNA-03

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Notes: